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DRUG DISCOVERY

Antifungal activity of *Piper betel* plants in Malaysia

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ABSTRACT

Piper betel plants and 5 strains of medically important fungi were selected for this study. Antifungal susceptibility test was performed to screen the antifungal activity of this plant against the selected fungi. *Piper betel* showed to possess antifungal property against 4 out of 5 strains of the fungus. Solid Phase extraction (SPE) technique was applied to *Piper betel* to achieve initial separation of active antifungal compound in the form of methanol fractions. These fractions were tested for their antifungal property. *Piper betel* showed the best antifungal activity against *A. flavus*.

Key words: antifungal activity, Solid phase extraction, *Piper betel. A. flavus*

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1. INTRODUCTION

There are many plant species of medicinal importance in Malaysia. There are more than 10,000 species of plants in Malaysia (Burkill et al. 1966). From the mystical ancient medication technique until today's high technology-oriented therapy, many of these local Malaysian plants have been widely used and scientifically tested as well to explore new therapeutic properties of these plants. Our main aim was to explore antimicrobial compound in the plants. Although many scientific studies have been conducted in order to unleash the mystery behind the medicinal plants, yet gaps remain which need to be completed. It seems that the antifungal activity of certain plant species has not been satisfactorily explored. There are about 100,000 species of fungus present in the environment and more than 100 of them are pathogenic in humans (Keeler and Tu, 1991). Our previous study showed that *Piper betel* plant has a degree of antifungal activity which prompted us to carry out further research.

2. MATERIALS AND METHODS

The *Piper betel* plants used in this study were collected from different states of Malaysia (Selangor, Perak, and Penang). Most of the species are wild vegetations in Malaysian forests and can be found even on the roadside. They are also found in local nurseries, market places etc. Five types of medically important fungi were chosen based on their ability to infect human. They were *Aspergillus flavus, Candida albicans, Microsporum canis, Trichophyton mentagrophytes* and *Trichophyton rubrum.* The chosen fungi were cultured on Sabouraud's Dextrose agar (SDA) and KOH preparation was used for microscopy. SDA is a standard culture media for fungus. Collected plant species were processed for extraction. Plants were dried; powdered, macerated and crude extracts were collected using standard methanol extraction process. Discs were prepared using these crude extracts for antifungal susceptibility test. All the discs prepared from crude plant extracts were tested for their antifungal activity. Standard Disc Diffusion method (Koneman, 2000) was used for this purpose. Solid Phase Extraction (SPE) technique (Duke, 1992) was applied in order to predict the location of the active antifungal compound using methanol.

3. RESULTS

3.1. Antifungal susceptibility test

Antifungal susceptibility test (using the original plant extracts without separation), was carried out using the disc diffusion method. *Piper betel* showed more or less antifungal activity against all the fungi tested. The diameter of the zone of inhibition were 16 mm against *A. flavus*, 8 mm against *C. albicans*, 13 mm against *M. cannis*, 12 mm against *T. mentagrophytes* and 15 mm against *T. rubrum*.

3.2. Fractionation

P. betel was separated into different methanol fractions and was tested against *A. flavus*. Methanol fractions were pipetted onto blank discs with the ratio of 100 µl of the fraction per disc. After 24 hours of incubation at 30^0C with the discs, clearing zones of 7 mm and 13 mm in diameter were produced by 40% and 50% methanol fractions,

respectively. Another series of fractionation was run on the same plant using different concentration of methanol (30%, 70%, 90%) and was tested against *A. flavus*. Also 40%, 50% and 60% methanol fractions of *P. betel* were tested against the other 4 species of fungus. Twelve mm diameter of inhibition zone was produced against *T. rubrum*, 10 mm against *T. mentagrophytes*, 10 mm against *M. canis* and 9 mm on *C. albicans*. *P. betel* plants collected from different locations showed the similar findings.

4. DISCUSSION

Fungal infections are very common in Malaysia, but the antifungal-related study on plant species is rare in Malaysia. It is important to study every aspect and every detail of the plant species known to have medicinal values. In this study, antifungal property of the *Piper betel* plant was examined. For this purpose, Sabouraud's Dextrose Agar (SDA) was used for culture and susceptibility test. Production of inhibition or clearing zone was carefully observed from time to time. A clearing zone of 10 mm was chosen as the cutoff point to indicate the presence of significant antifungal property of the plant fractions. Safety and sterility measures throughout the experiments were maintained to obtain significant results, to avoid contamination and infection as well. Different size of zones of inhibition was observed when different extracts were assayed. Antifungal susceptibility test (using the original plant extracts without separation) showed that *Piper betel* plant species contains positive activity towards the fungi or has the ability to suppress fungal growth. *Piper betel* was reported to have antibacterial activity (Fathilah et al. 2006) but its antifungal activity has never been explored before us. However, *P. betel* extract was also found to reduce acid producing properties of certain bacteria (Nalina et al. 2007). In this study, *P. betel* showed antifungal activity towards the maximum number of fungi tested. *P. betel* produced a diameter of clearing zone of 16 mm against *A. flavus*, 8 mm against *C. albicans*, 13 mm against *M. cannis*, 12 mm against *T. mentagrophytes* and 15 mm against *T. rubrum.* *P. betel's* antifungal activity against *C. albicans* was the least (8mm) compared to other fungi tested. Subsequently, *P. betel* was selected for fractionation and was separated into different methanol fractions as it showed significant antifungal value in the preliminary antifungal susceptibility test. After 24 hours incubation of its 50% methanol fraction at 30°C, the result showed a clearing zone of 13 mm in diameter (6 mm larger than the 40% fraction's inhibition zone, 3 mm less than the inhibition zone produced by the original extract) against *A. flavus*. The results suggest that 50% methanol fraction of *P. betel* has a significant antifungal property.

5. CONCLUSION

In summary, *Piper betel* has shown to contain the antifungal activity among all the plant fractions so far tested, especially on *A. flavus*. The antifungal activity was found to be highest in the 50% methanol fraction of *P. betel*. The Disc Diffusion method can be used to screen antifungal activity of the plant. Methanol extracts and Solid Phase Extraction can be applied to fractionate and isolate the active antifungal principles of the plants.

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REFERENCES

1. Burkill IH, Hanif M. Malay Village Medicine. Book, 1966. The Gardens Buletin Straits Settlement
2. Duke JA. Handbook of phytochemical constituents of GRAS herbs and other economic plants, *Boca Raton, FL, CRC Press*. 1992
3. Fathilah AR, Rofinah YO, Zubaidah HR. The effect of *Piper betel* and *Psidium guajava* extracts on the cell surface hydrophobicity of selected early settlers of dental plaque. *J Oral Sci* 2006, 48, 71-75
4. Keeler RF, Tu AT. Toxicology of Plant and Fungal Compounds in: Handbook of Natural Toxins, Vol. 6, 1991. Marcel Dekker, Inc. NY, 665 pp
5. Koneman WE. *Introduction to Diagnostic Microbiology*. 2000
6. Nalina T, Rahim ZHA. The crude aqueous extract of *Piper betel* and its antibacterial effect towards Streptococcus mutants. *Am J Biotech Biochem* 2007, 3, 10-15

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